MNA 2013 WORKSHOP:
EXPRESSION OF AXON GUIDANCE GENES IN THE PROSOMERE 1

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INTRODUCTION

The path that a navigating axon takes in the proximities of the midline it is determinate by the way its growth cone integrates a variety of signals present in the extracellular matrix and in the membrane of neighboring cells. The interaction with these molecules produces attraction, repulsion or a change in directionality by modifications in the growth cone cytoskeleton.

The family of gene x and its ligands are translated into transmembrane proteins which mediate bidirectional signaling within adjacent cells, activating signal transduction pathways in both ways, the cells that present the ligands and the ones that express the x protein. These signals modulate the dynamic of the cytoskeleton, affecting the cell morphology, motility and adhesion. It has been describe that this family participates in multiple morphogenic processes, including gastrulation, segmentation, axon guidance, fasciculation and cell migration along the neural tube.

Previous studies shown the presence of gene x in the dorsal zone of the diencephalon during early chick developmental stages, however it has not been describe the precise localization nor the relation of its expression with the formation of the posterior commissure (PC), the most important hallmark of the prosomere 1 (P1). Studies in our laboratory have elucidated the expression of this gene in the diencephalic roof plate (DRP) among different stages of development, and a correlation between the presence of SCO-spondin (axon guidance molecule), x gene expression and the formation of the PC has been achieved. Therefore during this project we aimed to study the expression of gene x during mammalian development using as tool Allen Brain mouse developmental atlas to asses if this pattern its evolutionary conserved.

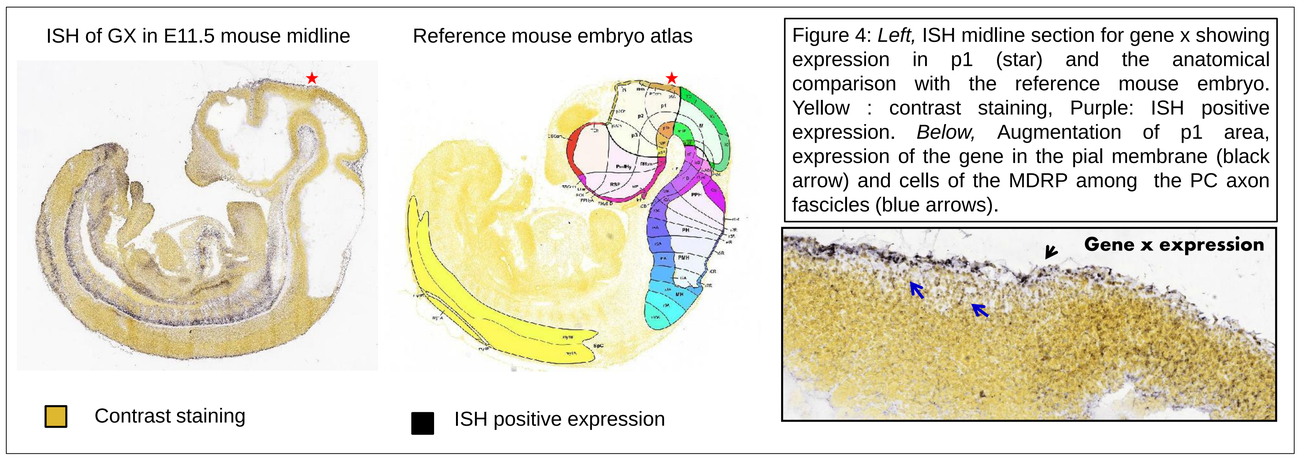


Figure 4: *Left,* ISH midline section for gene x showing expression in p1 (star) and the anatomical comparison with the reference mouse embryo. Yellow : contrast staining, Purple: ISH positive expression. *Below,* Augmentation of p1 area, expression of the gene in the pial membrane (black arrow) and cells of the MDRP among the PC axon fascicles (blue arrows).

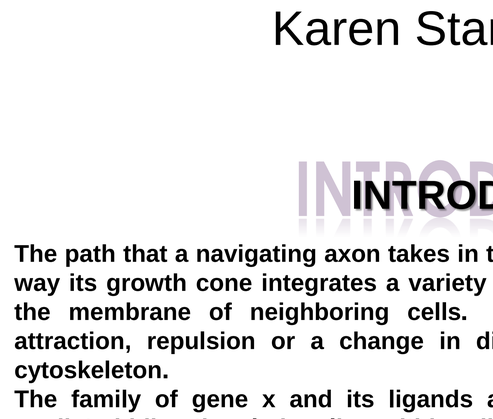


Figure 1: Expression of tubulin βIII in chick brain embryos. **A y B**: Sagittal sections of chick brain embryos stage HH17 and HH23 respectibly, immunostained with anti-tubulina β III antibody. **A' y B**' augmentation of the regions framed in A and B, where we can identify the axons that begin to form the posterior commissure (PC), localize above the diencephalic roof plate (DRP).

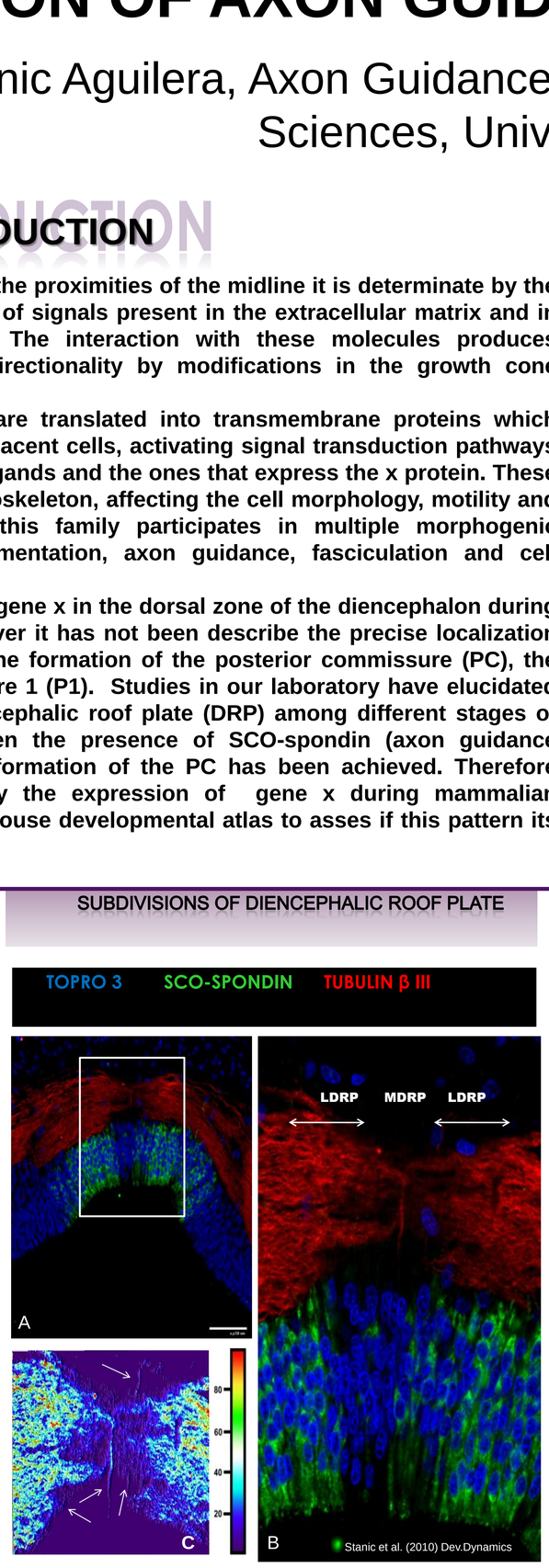


Figure 2:
A: Expression of SCO-spondin and tubulin β III in coronal sections of the DRP of HH32 chick embryo. **B**: augmentation of region framed in A. Results show the subdivisions of the diencephalic roof plate into a medial zone (MDRP) in where the axons are defasciculated and with different orientations, and two lateral zones (LDRP) where the axons are highly fasciculated. C: Pseudocolor image of axons immunostained with anti-tubulin β III, showing defasciculated axons in the midline and the change in direction in some of them (arrows).

Are axon guidance molecules expressed during early chick P1 development conserved in mammals?

RESULTS

ISH SAGITTAL SECTIONS OF GENE X EXPRESSION IN E11.5 MOUSE EMBRYOS

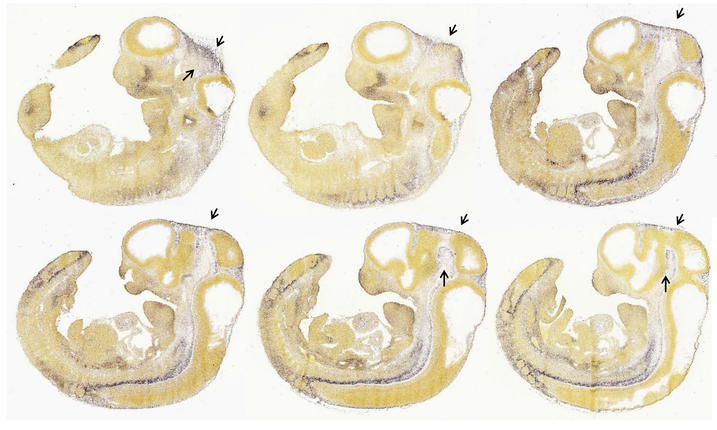


Figure 3: Allen Mouse Brain developmental atlas sections for gene x visualized by ISH. Sections from left to right , top to bottom advancing towards the midline zone. Expression of Gene X it is seen in the pial membrane, lateral alar plate, medial alar plate and dorsal roof plate (arrows).

EXPRESSION OF AXON GUIDANCE MOLECULES IN THE P1 DURING MOUSE DEVELOPMENT (E11.5)

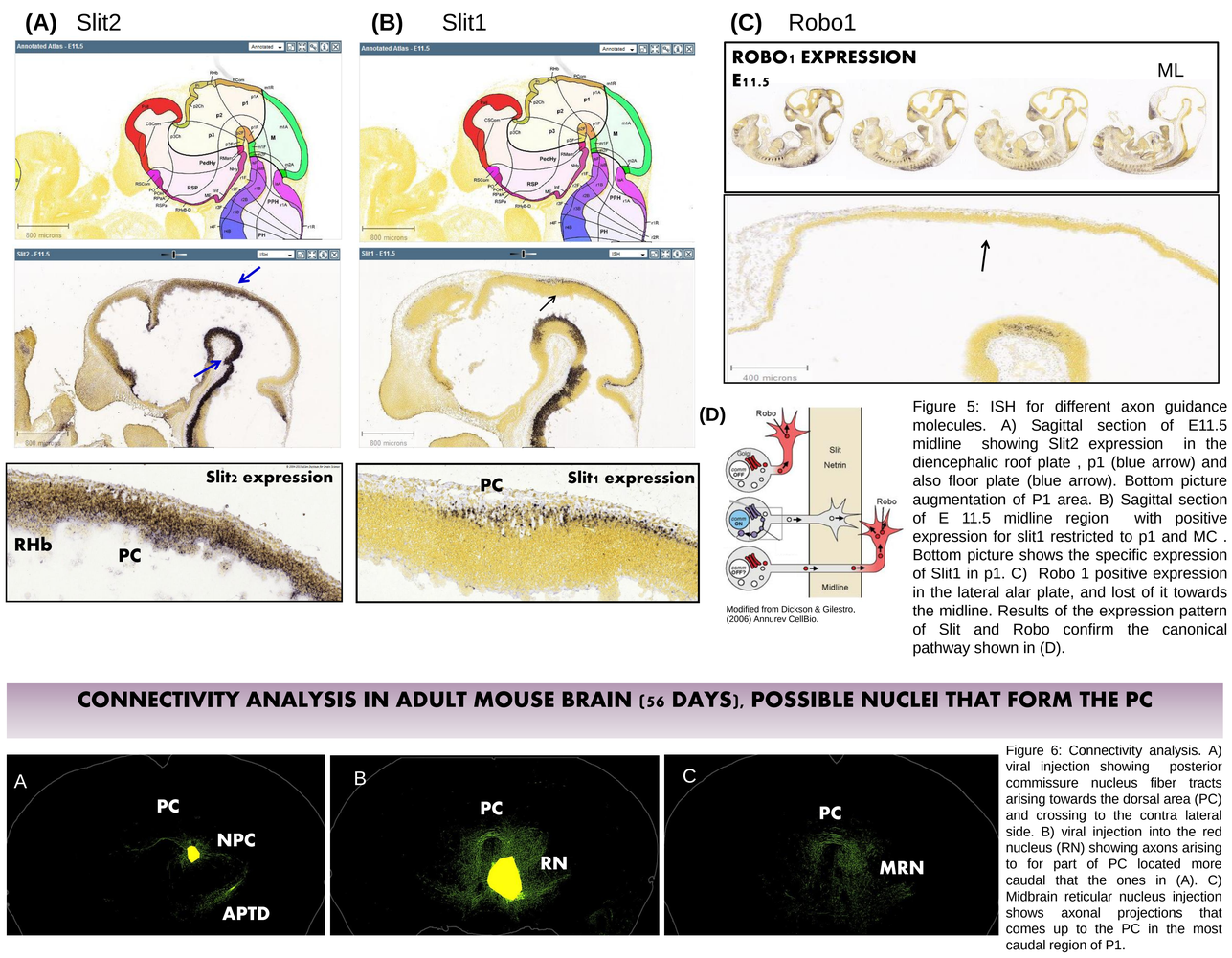


Figure 5: ISH for different axon guidance molecules. A) Sagittal section of E11.5 midline showing Slit2 expression in the diencephalic roof plate , p1 (blue arrow) and also floor plate (blue arrow). Bottom picture augmentation of P1 area. B) Sagittal section of E 11.5 midline region with positive expression for slit1 restricted to p1 and MC . Bottom picture shows the specific expression of Slit1 in p1. C) Robo 1 positive expression in the lateral alar plate, and lost of it towards the midline. Results of the expression pattern of Slit and Robo confirm the canonical pathway shown in (D).

CONNECTIVITY ANALYSIS IN ADULT MOUSE BRAIN (56 DAYS), POSSIBLE NUCLEI THAT FORM THE PC

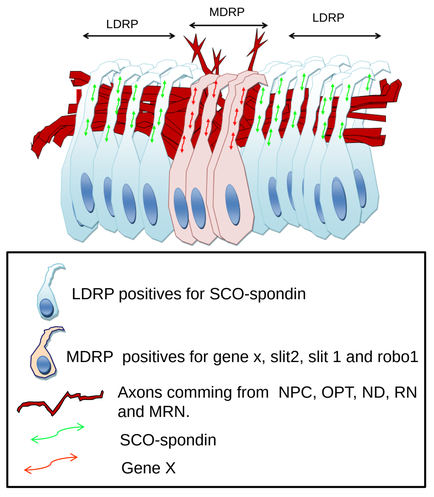
Figure 6: Connectivity analysis. A) viral injection showing posterior commissure nucleus fiber tracts arising towards the dorsal area (PC) and crossing to the contra lateral side. B) viral injection into the red nucleus (RN) showing axons arising to for part of PC located more caudal that the ones in (A). C) Midbrain reticular nucleus injection shows axonal projections that comes up to the PC in the most caudal region of P1.

CONCLUSIONS

-Gene X its conserved from avians to mammals with the same expression pattern, suggesting a similar mechanism throughout development in P1 region.

- Robo-Slit pathway expressed in E11.5 mouse embryos seems to be also present in P1 during avian development.

- PC have fiber components from the RN and MRN not describe yet in literature, which could explain the diversity of axon guidance molecules and the different effect of them on the axons that form this structure.



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